

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

Øklahoma Agricultural Experiment Station

There has been presented to the

Secretary of Agriculture

AN APPLICATION REQUESTING A CERTIFICATE OF PROTECTION FOR AN ALLEGED DISTINCT VARIETY OF SEXUALLY REPRODUCED, OR TUBER PROPAGATED PLANT, THE NAME AND DESCRIPTION OF WHICH ARE CONTAINED IN THE APPLICATION AND EXHIBITS, A COPY OF WHICH IS HEREUNTO ANNEXED AND MADE A PART HEREOF, AND THE VARIOUS REQUIREMENTS OF LAW IN SUCH CASES MADE AND PROVIDED HAVE BEEN COMPLIED WITH, AND THE TITLE THERETO IS, FROM THE RECORDS OF THE PLANT VARIETY PROTECTION OFFICE, IN THE APPLICANT(S) INDICATED IN THE SAID COPY, AND WHEREAS, UPON DUE EXAMINATION MADE, THE SAID APPLICANT(S) IS (ARE) ADJUDGED TO BE ENTITLED TO A CERTIFICATE OF PLANT VARIETY PROTECTION UNDER THE LAW.

NOW, THEREFORE, THIS CERTIFICATE OF PLANT VARIETY PROTECTION IS TO GRANT UNTO THE SAID APPLICANT(S) AND THE SUCCESSORS, HEIRS OR ASSIGNS OF THE SAID APPLICANT(S) FOR THE TERM OF TWENTY YEARS FROM THE DATE OF THIS GRANT, SUBJECT TO THE PAYMENT OF THE REQUIRED FEES AND PERIODIC REPLENISHMENT OF VIABLE BASIC SEED OF THE VARIETY IN A PUBLIC REPOSITORY AS PROVIDED BY LAW, THE RIGHT TO EXCLUDE OTHERS FROM SELLING THE VARIETY, OR OFFERING IT FOR SALE, OR REPRODUCING IT, OR PORTING IT, OR EXPORTING IT, OR CONDITIONING IT FOR PROPAGATION, OR STOCKING IT FOR ANY OF THE VE PURPOSE, OR CONDITIONING IT FOR PROPAGATION, OR STOCKING IT FOR ANY OF THE ABOVE OSE, OR USING IT IN PRODUCING A HYBRID OR DIFFERENT VARIETY THEREFROM, TO THE EXTENT TO BY THE PLANT VARIETY PROTECTION ACT. IN THE UNITED STATES SEED OF THIS VARIETY BE SOLD BY VARIETY NAME ONLY AS A CLASS OF CERTIFIED SEED AND (2) SHALL CONFORM TO THE NUMBER OF THE RIGHTS. (84 STAT. 1542, AS AMENDED, 7 U.S.C. 2321

BERMUDAGRASS

'Riviera'

In Testimony Whereof, I have hereunto set my hand and caused the seal of the Hunt Antiety Protection Office to be affixed at the City of Washington, D.C. this twenty-seventh day of April, in the year two thousand and five.

Attest:

HM J. Commissioner

Plant Variety Protection Office Agricultural Marketing Service Secretary of Agriculture

CAPACITY OR TITLE

Asst. Director, OAES Apri ST-470 (02-10-2003) designed by the Plant Variety Protection Office using Word 2000. Repla

DATE

April, 7, 2003

DATE

CAPACITY OR TITLE

GENERAL: To be effectively filed with the Plant Variety Protection Office (PVPO), ALL of the following items must be received in the PVPO: (1) Completed application form signed by the owner; (2) completed exhibits A, B, C, E; (3) for a seed reproduced variety at least 2,500 viable untreated seeds, for a hybrid variety at least 2,500 untreated seeds of each line necessary to reproduce the variety, or for tuber reproduced varieties verification that a viable (in the sense that will reproduce an entire plant) tissue culture will be deposited and maintained in an approved public repository; (4) check drawn on a U.S. bank for \$3,652 (\$432 filing fee and \$3,220 examination fee), payable to "Treasurer of the United States" (See Section 97.6 of the Regulations and Rules of Practice.) Partial application will be held in the PVPO for not more than 90 days, then returned to the applicant as unfilled. Mail application and other requirements to Plant Variety Protection Office, AMS, USDA, Room 401, NAL Building, 10301 Baltimore Avenue, Beltsville, MD 20705-2351. Retain one copy for your files. All items on the face of the application are self explanatory unless noted below. Corrections on the application form and exhibits must be initialed and dated. DO NOT use masking materials to make corrections. If a certificate is allowed, you will be requested to send a check payable to "Treasurer of the United States" in the amount of \$432 for issuance of the certificates will be issued to owner, not licensee or agent.

Plant Variety Protection Office Telephone: (301) 504-5518 FAX: (301) 504-5291

Homepage: http://www.ams.usda.gov/science/pvpo/pvp.htm

ITEM

18a. Give:

- (1) the genealogy, including public and commercial varieties, lines, or clones used, and the breeding method;
- (2) the details of subsequent stages of selection and multiplication:
- (3) evidence of uniformity and stability, and
- (4) the type and frequency of variants during reproduction and multiplication and state how these variants may be identified
- 18b. Give a summary of the variety's distinctness. Clearly state how this application variety may be distinguished from all other varieties in the same crop. If the new variety is most similar to one variety or a group of related varieties:
 - (1) identify these varieties and state all differences objectively;
 - (2) attach statistical data for characters expressed numerically and demonstrate that these are clear differences; and
 - (3) submit, if helpful, seed and plant specimens or photographs (prints) of seed and plant comparisons which clearly indicate distinctness.
- 18c. Exhibit C forms are available from the PVPO Office for most crops; specify crop kind. Fill in Exhibit C (Objective Description of Variety) form as completely as possible to describe your variety.
- 18d. Optional additional characteristics and/or photographs. Describe any additional characteristics that cannot be accurately conveyed in Exhibit C. Use comparative varieties as is necessary to reveal more accurately the characteristics that are difficult to describe, such as plant habit, plant color, disease resistance, etc.
- 18e. Section 52(5) of the Act requires applicants to furnish a statement of the basis of the applicant's ownership. An Exhibit E form is available from the PVPO.
- 19. If "Yes" is specified (seed of this variety be sold by variety name only, as a class of certified seed), the applicant MAY NOT reverse this affirmative decision after the variety has been sold and so labeled, the decision published, or the certificate issued. However, if "No" has been specified, the applicant may change the choice. (See Regulations and Rules of Practice, Section 97.103).
- 22. See Sections 41, 42, and 43 of the Act and Section 97.5 of the regulations for eligibility requirements.
- 23. See Section 55 of the Act for instructions on claiming the benefit of an earlier filing date.
- 21. CONTINUED FROM FRONT (Please provide a statement as to the limitation and sequence of generations that may be certified.)

Syn-1 generation seed from fields planted with vegetative propagules (sprigs) of the three parent plants may be classified as Foundation, Registered or Certified. Foundation and Registered seed classes are for the specific purpose of establishing Certified class sod production fields by contractual agreement between the owner and licensee(s). Certified class seed will be the seed of regular commerce.

22. CONTINUED FROM FRONT (Please provide the date of first sale, disposition, transfer, or use for each country and the circumstances, if the variety (including any harvested material) or a hybrid produced from this variety has been sold, disposed of, transferred, or used in the U.S. or other countries.)

First sale of seed was April 15, 2002 by Johnston's Seed Co. Enid, OK. Sale of Riviera seed in 2002 was limited to a few hundred pounds.

23. CONTINUED FROM FRONT (Please give the country, date of filing or issuance, and assigned reference number, if the variety or any component of the variety is protected by intellectual property right (Plant Breeder's Right or Patent).)

70. C

NOTES: It is the responsibility of the applicant/owner to keep the PVPO informed of any changes of address or change of ownership or assignment or owner's representative during the life of the application/certificate. There is no charge for filing a change of address. The fee for filing a change of ownership or assignment or any modification of owner's pame is specified in Section 97.175 of the regulations. (See Section 101 of the Act, and Sections 97.130, 97.131, 97.175(h) of the Regulations and Rules of Practice.)

To avoid conflict with other variety names in use, the applicant must check the appropriate recognized authority. For example, for agricultural and vegetable crops, contact: Seed Branch, AMS, USDA, Room 213, Building 306, Beltsville Agricultural Research Center-East, Beltsville, MD 20705. Telephone: (301) 504-8089. http://www.ams.usda.gov/lsg/seed.htm

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0581-0055. The time required to complete this information collection is estimated to average 3.0 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

The U.S. Department of Agriculture (USDA) prohibits discrimination in all its programs and activities on the basis of race, color, national origin, gender, religion, age, disability, sexual orientation, marital or family statupolitical beliefs, parental status, or protected genetic information. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (Braille, large print, audiotape, etc.) should contact USDA's TARGET Center at 202-720-2600 (voice and TDD).

To file a complaint of discrimination, write USDA, Director, Office of Civil Rights, Room 326-W, Whitten Building, 14th and Independence Avenue, SW, Washington, DC 20250-9410 or call 202-720-5964 (voice and TDD). USDA is an equal opportunity provider and employer

TDD). USDA is an equal opportunity provider and employer.

ST-470 (02-10-2003) designed by the Plant Variety Protection Office with Word 2000. Replaces former versions of ST-470, which are obsolete.



18a. Exhibit A. Origin and Breeding History of the Variety.

'Riviera' (experimental designation 'OKS 95-1'), Cynodon dactylon var. dactylon, is a tetraploid (2n=4x=36 chromosome) synthetic variety produced by the polycrossing of three clonal parent plants. The three parents were selected in spring 1995 from a breeding population grown as individual space-planted plants for purposes of cyclic selection. The parent plants were selected on the bases of visual and/or measured assessments of characters conditioning turf quality, transition zone adaptation, and seed production. The three clonal parent plants were planted in a field polycross nursery on the Agronomy Research Station, Stillwater, Oklahoma in July 1995. Evaluations of Riviera have been from plantings established using Syn-1 seed produced from this block.

The breeding population from which the three Riviera parent plants were selected was developed in 1994 using the following bermudagrasses.

Accession (A) Number or	
other ID	Origin
A12388	Seed collected from site near Zhaoqing City, Guangdong
	Province, Peoples Republic of China, 8/22/93
A12390	Seed collected from Ziaoshiao Grass Res. Stn., Yunnan
	Province, PRC, 8/27/93
A12392	Seed collected from site in Xindian County, Yunnan
	Province, PRC, 8/28/93
A12396	Seed collected from site near Kunming, Yunnan Province,
	PRC, 8/30/93
A12398	Seed collected from site near Kunming, Yunnan Province,
	PRC, 8/30/93
A12399	Seed collected from site near Kunming, Yunnan Province,
	PRC, 8/30/93
A12402	Seed collected from site near Kunming, Yunnan Province,
	PRC, 8/30/93
A12403	Seed collected from site near Kunming, Yunnan Province,
	PRC, 8/30/93
A12407	Seed collected from site near Emeishan City, Sichuan
	Province, PRC, 9/3/93
A12410	Seed collected near Nanjing, Jiangsu Province, PRC,
	9/6/93
CdaRS C2	Recurrent selection cycle 2 of an Oklahoma State
	University breeding population first formulated in 1982.

The population was formed by growing 20 plants of each of the above indicated bermudagrasses in an isolated field polycross nursery planted on the Oklahoma State University Agronomy Research Station at Stillwater, OK in early spring 1994. Plants of each bermudagrass accession were arranged in the polycross to facilitate random

crossing. A portion of the seed harvested from the field polycross nursery in early July 1994 was germinated soon after harvest and 1024 resultant plants were transplanted from the greenhouse to a field selection nursery in mid-August 1994. It was from this nursery that the three Riviera parent plants were selected in late June 1995. No other commercial variety has been developed to date from the breeding population from which Riviera was derived.

Riviera is uniform and stable within defined limits of natural variation existing within the Syn-1 generation. Only the Syn-1 generation seed generation is allowed. Syn-1 generation seed is produced from plantings of approximately equal quantities of Foundation class clonal propagules (sprigs) of the three parent plants. The Syn-1 generation seed may be classified as Registered class, or downgraded to the Certified class. Registered class seed can be used only for the specific purpose of establishing plantings from which Certified class sod is to be harvested and marketed. Certified seed is the seed of regular commerce. The three clonal parent plants are non-inbred and therefore genetically heterozygous. Strong self-incompatibility (≤ 2% seed set from selfpollination) of the parent plants ensures seed of predominantly hybrid origin. Random mating (polycrossing) among the three parent plants produces offspring that are phenotypically heterogeneous, but whose mean value for a given trait is stable and predictable. The Syn-1 generation plants that are most distinctly different in appearance from the norm due to color, size, or texture are found within the tails of the statistically normally distributed population. Accordingly, these variant plants constitute less than 5% of the plants in the population. The extent (range) of variation found among plants for the important descriptors is addressed in Exhibit B. No off-type plants resulting from crossing with other varieties, or from mechanical contamination with other varieties, have been detected.

18b. Exhibit B. Statement of Distinctness.

'Riviera' is most similar to 'Jackpot' and 'Mirage' in adaptation and morphological characteristics. Comparisons of Riviera with Jackpot and Mirage for descriptive characteristics are summarized in Tables B1 through B18. Four conspicuous morphological differences between Riviera and both Jackpot and Mirage are as follows:

- 1. Mean leaf blade color rating of Riviera (8.4) is greater than that of Mirage (6.5) and Jackpot (6.4) indicating Riviera to have darker green color (Table B7).
- 2. Mean number of racemes per inflorescence of Riviera (4.2) is less than that of Jackpot (5.0) and Mirage (5.0) (Table B12).
- 3. Mean head exertion length of Riviera (19.1 mm) is less than that of Jackpot (33.3 mm) and Mirage (26.3 mm) (Table B14).
- 4. Mean peduncle length of Riviera (81.3 mm) is less than that of Jackpot (93.2 mm) and Mirage (91.4 mm) (Table B15).

Table B7. Mean leaf blade color ratings for Riviera, Mirage and Jackpot seed-propagated turf bermudagrass cultivars based on five measurements from each of 60 field grown plants in 1999 and 2000[†]. Rating scale was 1 to 9 where 1=light green and 9=dark green. Test located on the Agronomy Research Station, Stillwater, OK.

		Leat	Blade Color Rat	ings	
		Means		Rai	nges
Cultivar	1999	2000	2 Yr. Mean	1999	2000
	~~~~~~~~~~		Rating		
Riviera	8.2	8.6	8.4	6-9	6-9
Mirage	6.6	6.4	6.5	6-9	5-9
Jackpot	6.3	6.5	6.4	6-7	6-7
5% LSD*	1.2	1.1	0.8		

Table B12. Mean number of racemes per inflorescence for Riviera, Mirage and Jackpot seed-propagated turf bermudagrass cultivars based on five measurements from each of 60 field grown plants in 1999 and 2000[†]. Test located on the Agronomy Research Station, Stillwater, OK.

		<u> </u>	acemes/Infloresc	ence	
		Means	t i p	Ra	nges
Cultivar	1999	2000	2 Yr. Mean	1999	2000
- National Additional Philadelphia			No		
Jackpot	5.0	5.0	5.0	3-6	3-6
Mirage	5.0	5.0	5.0	4-6	4-7
Riviera	4.1	4.3	4.2	3-5	3-5
5% LSD*	0.6	0.5	0.5		

Table B14. Mean head exsertion length (mm) for Riviera, Mirage and Jackpot seed-propagated turf bermudagrass cultivars based on five measurements from each of 60 field grown plants in 1999 and 2000[†]. Measurements were from base of inflorescence to the flag leaf. Test located on the Agronomy Research Station, Stillwater, OK.

		Head	l Exsertion Lengt	h (mm)	
	4-9-9	Means		Ra	nges
Cultivar	1999	2000	2 Yr, Mean	1999	2000
			mm		
Jackpot	32.4	34.2	33.3	0-96	0-94
Mirage	26.8	25.8	26.3	1-84	0-80
Riviera	17.6	20.6	19.1	0-75	0-79
5% LSD*	5.2	5.0	4.9		

Table B15. Mean peduncle length (mm) for Riviera, Mirage and Jackpot seed-propagated turf bermudagrass cultivars based on five measurements from each of 60 field grown plants in 1999 and 2000[†]. Measurements taken were from base of whorl to first node. Test located on the Agronomy Research Station, Stillwater, OK.

		P	eduncle Length (n	nm)	
		Means		Rai	nges
Cultivar	1999	2000	2 Yr. Mean	1999	2000
			mm		
Jackpot	94.9	91.5	93.2	30-165	11-150
Mirage	89.7	93.1	91.4	47-162	50-160
Riviera	83.1	79.5	81.3	16-84	21-86
5% LSD*	5.7	5.6	5.5		

REPRODUCE LOCALLY. Include form number and date on all reproductions.

Form Approved - OMB No. 0581-0055

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> U. S. DEPARTMENT OF AGRICULTURE AGRICULTURAL MARKETING SERVICE SCIENCE AND TECHNOLOGY PLANT VARIETY PROTECTION OFFICE BELTSVILLE, MD 20705

EXHIBIT C (Bermudagrass)

OBJECTIVE DESCRIPTION OF VARIETY BERMUDAGRASS (Cynodon spp.)

			•
NAME OF APPLICANT(S)		<u> </u>	FOR OFFICIAL USE ONLY
Oklahoma Agricultur	al Experiment Station		PVPO N 2 TO 0 3 0 0 2 2 1
ADDRESS (Street and No. or R.F.D. No., C	•	,	VARIETY NAME
139 Agricultural Ha	11	•	
Stillwater, OK 740	/8-6019		Riviera TEMPORARY OR EXPERIMENTAL DESIGNATION
•			OKS 95-1
(e.g. 0/9/9 or 0/9/) when no including numerical measu must be made under the sai unclipped spaced plants the unclipped plots or individual plants when measured). A	umber is either 99 or less or 9 or less. It rements, should represent those that are ne conditions. Append all pertinent contractions are represent the application variety, the	The symbol "A" indicates of TYPICAL for the variety omparative trial and evaluation most similar variety, and course. Data should be obtain	tion data. Measured data should be for one standard cultivar, or replicated ned from mature plants (specify age of
	STANDARD CULTIVARS Use culti	vars from same species and	d ploidy level
1 = Seeded Common	4 = Tifway	7 = Coastal	10 = other (Specify species)
2 = Guymon	5 = Tifgreen	8 = Coastcross-1	Jackpot, Cynodon dactylor
3 = Mirage	6 = Midiron	9 = Giant	Jackpot, Cynodon daetyfon
varieties that are adapted to	your area. One of the comparison vari	eties must be the most sim	
MSV 1. Mirage	Variety 2. <u>Jackpot</u>	V	ariety 3.
1 = C. dac $2 = C. dac$ $3 = C. trac$ $4 = C. dac$	ison varieties for use below - use varientlylon var. dactylon tylon var. aridus nsvaalensis tylon X C. transvaalensis (Specify).	Is this an F	ication variety) hybrid? urf or forage use? or clonally propagated?
2. CYTOLOGY  3 6 2n Chron	nosome Number		

Ploidy				00300221
1 = diploid 2 = tetraploid	•			
3 = triploid		3 603137 1.4	Comparison Variety 2	Comparison Variety 3
4 = Other (Specify)	Application Variety	MSV Variety 1		
	2	2	2	
		Tastade 2 Not Adented	· 3 = Adapted)	
<b>3. ADAPTATION:</b> ( 0=	Not tested; 1= Inadequately	Tested, 2= Not Adapted	, <i>5</i> = 1 kdapte 5)	
1 Northwest	1 North Central	1 Northeast	Other	
3 West Central	3 Central	3 East Central	Other	•
3 Southwest	3 South Central	3 Southeast	Other	· ·
	•			•
4. RHIZOMES	1			
1 = None (Coastcross -1) 4 = Weakly Rhizomatou	s (Coastal)			•
6 = Moderately Rhizoma	atous (Common)		•	
9 = Heavy Rhizomatous				
	9	9	9	
`				
Amount of spread in 1	year cm			
	90	90	90	
	growing conditions: Still to mation (cool temperature). Ex			ertilized & irrigated
Anthocyanın pigmenta	mon (coor temperature). 157	dimproof probably in the	•	
or	d niamontation			
Percent of plants with	anthocyanin pigmentation		;	
	60	<del></del>		<u> </u>
Cu 1	th cm. Measure from betwe	en 3 rd and 4 th fully extend	ded nodes from apical mer	istem.
Stoion internoue lengt	III CIII. Wicasare from ootho			
	4.20	3.97	2.79	
Cu 1 do diom	neter mm. Measure from ce	nter of 3 rd fully extended	internode from apical me	ristem.
Stolon internode dian			1.19	
	1.11	1.38	1.17	
	oints at a mature node. Re	ecommend 4 th node.		
Number of growing p	omis at a mature node. 100			•
	1.31	1.37	1.18	
- 10 111 - 1				
Specify which node w	as counted.	•	ur . ei	•
•	<u>4th</u>	4th	4th	
و د د د وهاد چې سا				
Length of longest stol	on cm		07.0	•
	37.3	42.5	27.0	

7	n	A	3	n	A	9	9	1
٠	•	1 F	٠,5	3/	1,7	( <del>-</del>	See	- 23

	Application Variety	MSV Variety 1	Comparison Variety 2	Comparison Variety 3
	194.9	187.9	145.9	
6. LEAF BLADE:				
Color 1 = Light Green (Baysho 3 = Light Medium Green 5 = Medium Green (Guy 7 = Medium Dark Green	ı, vmon),			
9 = Dark Green (Tifgree				
	7	5	5	
Other Color 1 = Bluegreen (Tifdwarf 2 = Grey Green 3 = Other (specify)	f, No Mow)	·		
Percent plants with oth	ner color			
Width Class 1 = Very Coarse (Coaste 3 = Coarse (Midland, G 5 = Medium (Seeded Co	uymon)			
7 = Fine (Tifway) 9 = Very Fine (Tifgreen	7	5	5	
Leaf length cm. Measu	re longest leaf at third no	de below apical meristem	on main upright tiller.	<i>:</i>
Leaf length one mouse	4.79	4.94	4.37	
Lasf width mm Mage	prement on 3 rd or 4 th leaf l	elow apical meristem. M	leasure width at widest part a	bout 1 cm from base.
Lear with time weast				•
	<u></u>	2.07		
Flag leaf length cm	2.17	2.49	2.31	
Flag leaf width mm. 1	Measure width at widest p	art or about 1 cm from ba	se.	
B			0.99	
		,		
Flag leaf sheath length		<i>c t</i> . 9	60 8 °	
			60.8	
Leaf width mm (later			at 4 th node from tip of stolon.	· ·
•	2.22	2.59	2,55	
	•		•	
Leaf length cm (latera	al leaves). Measure the le	ongest part of largest leaf	at 4 th node from tip of stolon.	

•	
eaf blade hair number	(use $1 = absent$ ; several; $9 = abundant$ ).

Leaf blade hair number	(use $1 = absent$ ; several;	y = aoundant).		
	Application Variety	MSV Variety 1	Comparison Variety 2	Comparison Variety 3
	Several	Several	Several	
Leaf blade hair length (	use 1 = absent; 5=short;	9 = very long).		
year Marie Tonger (		5	5	
	<del></del>			
eaf sheath hair numbe			g	
	Several	Several	Several	
eaf sheath hair length	(use 1 = absent; 5=short	t; 9 = very long).		
	5	5	5	
Leaf collar hair number				
Leaf collar hair length	(use 1 = absent; 5=short	; 9 = very long).		
7. INFLORESCENCE	(Specify site, season, ar	nd growing conditions).		
Inflorescence length cm	. The length of the race	mes on the inflorescence.		
	Application Variety	MSV Variety 1	Comparison Variety 2	Comparison Variety
	4.54	4.48	4.36	
Number of racemes per	inflorescence.			·
	4.2	5.0	5.0	
Number of whorls per	inflorescence.	•		
Manibel of whoms per	1.0	1.02	1.0	
		C. L. F. Flarescance		
Percent of plants with	more than one whorl o	f branches/inflorescence.		
		<u>~</u>	_	
Percent of inflorescence	es with more than 1 wl	norl.		
		<1	< 1	
Spikelets per raceme.				
Opinicies per raceme.	41.1	41.3	42.3	·
Spikelet spacing on rad		M		
Spikelet spacing on rac	ceme mm Measured fro	m bottom 1/3 of spike.	_	

	Application Variety	MSV Variety 1	Comparison Variety 2	Comparison Variety 3
4 - C - 1				A A Service - A Constitution of the Constituti
ercent of plants with s	spike anthocyanii			
				-
ligma color % plants	with white stigmas. Mea	sure within 24 hours after	er anthesis.	
	90	10	50	
tigma color % plants	with light purple stigma	s. Measure within 24 ho	urs after anthesis.	
·	5	20	30	
tigma color % plants	with purple stigmas. Me	easure within 24 hours at	fter anthesis.	
	5	70		
nther color % plants	with purple anthers. M	easure within 24 hours a	fter anthesis.	
	80	65	100	
anther color % plants	with yellow anthers. Me	easure within 24 hours af	ter anthesis.	
:	20		0	400
	· ·			
		inflorescence to the fla	a leaf	· · · · · · · · · · · · · · · · · · ·
lead exertion cm. Me	easure from the base of the			
:	1.91	2.63	3.33	
÷	1.91  Measure internode from ba	2.63 se of whorl to first node.		· · · · · · · · · · · · · · · · · · ·
:	1.91	2.63	3.33	
Peduncle length cm. M	1.91  Measure internode from ba  8.13	2.63 se of whorl to first node.		
Peduncle length cm. M	1.91  Measure internode from ba  8.13	2.63 se of whorl to first node.		
Peduncle length cm. M First internode length	1.91  Measure internode from ba  8.13  cm.	2.63 se of whorl to first node. 9.14 5.07	9.32	
Peduncle length cm. M First internode length	1.91  Measure internode from ba  8.13  cm.  3.91	2.63 se of whorl to first node. 9.14 5.07	9.32	
Peduncle length cm. M First internode length Flag leaf sheath length	1.91  Measure internode from ba  8.13  cm.  3.91  h cm. Measure from node	2.63 se of whorl to first node.  9.14  5.07 to flag leaf base.  6.48	9.32 5.08	
Peduncle length cm. M First internode length Flag leaf sheath length	1.91  Measure internode from ba  8.13  cm.  3.91  h cm. Measure from node  6.22  (Specify site, time, growing)	2.63 se of whorl to first node.  9.14  5.07 to flag leaf base.  6.48  ng conditions).	9.32 5.08	extension for measureme
Peduncle length cm. M First internode length Flag leaf sheath length	1.91  Measure internode from ba  8.13  cm.  3.91  h cm. Measure from node  6.22  (Specify site, time, growing)	2.63 se of whorl to first node.  9.14  5.07 to flag leaf base.  6.48  ng conditions).	9.32 5.08	extension for measureme
Peduncle length cm. M First internode length Flag leaf sheath length B. PLANT HEIGHT Plant height cm. Mea	1.91  Measure internode from ba  8.13  cm.  3.91  h cm. Measure from node  6.22  (Specify site, time, growing sure at maturity, using the	2.63 se of whorl to first node.  9.14  5.07 to flag leaf base.  6.48  ng conditions). stallest inflorescence per  55.12	9.32  5.08  6.08  plant and hold out to furthest 42.23	extension for measureme

9. SEED, LEMMA, ANI	D GLUME: Use seed h	arvested from PVP nurse	ery, not commercial seed lots.	2003002
Glume length mm	Application Variety	MSV Variety 1	Comparison Variety 2	Comparison Variety 3
	1.6	·		
Glume width mm				
	0.2	· ·		
Lemma length mm				
	2.1			
Lemma width mm				•
	0.1			
Glume/lemma length ra	tio .			
	0.76			<u> </u>
Lemma keel hair numbo	er (use 1 = absent; 5=se	veral; 9 = many).		
	5			
Lemma keel hair length	use 1 = absent; 5=sho	rt; 9 = very long).		• .
Lemma margin hair nu	mber (use 1 = absent; 5	=several; 9 = many).		
- -			<u> </u>	
Lemma margin hair ler	ngth (use 1 = absent; 5=	short; 9 = very long).		
	5			· <u>· · · · · · · · · · · · · · · · · · </u>
Seed length mm (naked	l caryopses).		·	
	1			
Seed width mm (naked	caryopses).		1	
	0/4			
	<del></del>			
Explain if samples are b	lown and unhulled or hu	lled. <u>Blown and hu</u>	111ed	
Watcht of 100 cord mg				
Weight of 100 seed mg	23		· · · · · · · · · · · · · · · · · · ·	
Number of seeds per g	4450			
	4430			

#### 10. LOW TEMPERATURE TOLERANCE (Winter hardiness)

- 1 = Low or 100% injury (Coastcross-1, Common)
- 4 = Moderately Low (Coastal, Brazos)
- 6 = Moderately High (Tifway, Guymon, Tifdwarf)
- 9 = High or no injury (Midiron, Midland)

 Application Variety	MSV Variety 1	Comparison Variety 2	Comparison Variety 3
8	6	6	
•			

#### 11. DISEASES AND INSECTS

(0=Not Tested, 1=Susceptible, 2=Moderately susceptible, 3=Moderately resistant, 4=Resistant):

0	Brown patch (Rhizotonia solani)	0	Aphids
0	Dollar spot (Sclerotinia homoeocarpa)	0	Bermudagrass mite (Eriophyes cynodoniensis)
0	Fading out (Curvularia spp.)	0	Chinch bugs
3	Leafspot (Bipolaris spp.)	0	Ground pearl (scale)
-0	Rusts (Puccinia spp.)	0	Grubs
2	Spring Dead Spot (Pathogen indefinite)	0	Thrips
0	Zonate leafspot (D. gigantea)	0	Whitefly
	Other:		Other:

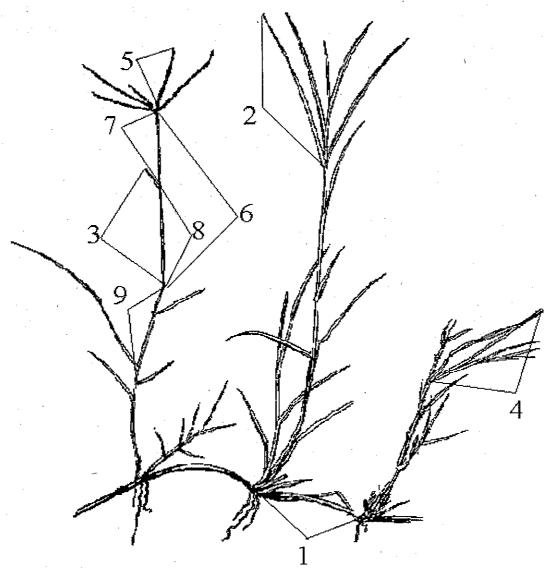
12. INDICATE THE SEED PROPAGATED VARIETY THAT MOST CLOSELY RESEMBLES THE APPLICATION VARIETY FOR THE FOLLOWING CHARACTERS: For each of the following characters, indicate the degree of resemblance by placing in the column marked "D.R." one of the following numbers.

- 1 = Application variety is less than comparison variety.
- 2 = Same as.
- 3 = More than, better, greater, darker, etc.

CHARACTER	VARIETY	D.R.
Rate of Spread	Mirage	2
Sod Density	Princess 77	2
Color	Guymon	2
Cold Tolerance	Guymon	2

13. SPECIFY LOCATION, GROWING CONDITIONS, AND EXPERIMENTAL DESIGN BELOW. Include location, age of plants, date of data collection (with daylength if possible), management conditions, experimental design etc.). Attach more paper if needed.

All measurements taken at the Agronomy Research Station, Okla. St. Univ., Stillwater, OK. Plants (60 of each) of Riviera, Yukon, Arizona Common, Mirage, Jackpot, and NuMex Sahara were planted in the field in 1998 on a Kirkland silt loam soil. Data were taken during the 1999 and 2000 growing seasons. Plots were fertilized and irrigated as needed to maintain a good growth environment. Plants were arranged in a randomized complete block experimental design with split plots. Whole plots were varieties and subplots were plants within variety. Four replications of 15 plants per rep were used. The developmental stage of plants at time of measurement varied with the character being measured. Most measurements were taken on plants near or at heading stage. During summer 2002, 20 plants each of Riviera and Princess 77 were grown in 6" diameter pots in the greenhouse and used to collect data on morphological characters. Plants were arranged in a randomized complete block design with 20 reps i.e. l plant/variety/rep.



### Bermuda grass (Cynodon dactylon)

- Stolon internode length
- 2. First fully extended leaf of upright growth
- 3. Flagleaflength
- 4. First fully extended leaf from tip of stolon5. Inflorescence length
- 6. Peduncie length7. Head exsertion
- Sheath length
- 9. First internode length

#### REFERENCE

Parker, Kittie F., An Illustrated Guide to Arizona Weeds. Drawings by Lucretia Breazeale Hamilton. Tucson, University of Arizona Press [1972]. xii, 338 p. illus.

18d. Exhibit D. Additional description of variety.

Riviera differs from selected seed-propagated turf bermudagrass varieties other than the two most similar varieties (Mirage and Jackpot) as follows:

- 1. Tolerance to low freezing temperatures is greater for Riviera ( $T_{mid} = -8.3$  °C) compared to Princess ( $T_{mid} = -6.9$  °C) (Table D1).
- 2. Riviera has faster spring greenup, a higher percentage of living ground cover in early spring, less winterkill, and lower winter injury than Princess, SWI-11, Transcontinental, Savannah, Southern Star, Blackjack, J-540, Majestic, Sydney, Sundevil II, Shangri La, Pyramid, Blue-Muda, Numex-Sahara, and Arizona Common seeded bermudagrass varieties as indicated by ratings from the 1997 National Turf Evaluation Program (NTEP) bermudagrass test (Table D2).
- 3. Riviera has better turfgrass quality than Princess, SWI-11, Transcontinental, Savannah, Southern Star, Blackjack, J-540, Majestic, Sydney, Sundevil II, Shangri La, Pyramid, Blue-Muda, Numex-Sahara, and Arizona Common seeded bermudagrass varieties as indicated by ratings from the 1997 NTEP bermudagrass test for Management Schedule B (Table D3). Riviera turfgrass quality is superior to all seeded bermudagrass varieties except Princess under Management Schedule A (Table D3).
- 4. Mean stolon internode diameter of Riviera (1.11 mm) is less than that of Guymon 92.04 mm), Yukon (1.35 mm), and Arizona Common (1.33 mm) seeded bermudagrass varieties (Table D4).
- 5. Mean stolon internode length of Riviera (41.98 mm) is shorter than that of Arizona Common (46.7 mm) and longer than that of Guymon (34.95 mm) and and Yukon (31.55 mm) (Table D5).
- 6. Mean number of growing points emanating from the 4th node of mature stolons of Riviera (1.31) is less than that of Arizona Common (1.21), Yukon (1.16) and Guymon (1.09) (Table D6).
- 7. Mean number of growing points emanating from the 4th node of mature stolons of Riviera (1.26) is less than that of Princess (1.56) (Table D7).
- 8. Mean stolon length of Riviera (194.9 mm) is less than that of Arizona Common (215.6 mm) and greater than that of Guymon (171.1 mm) and Yukon (146.3 mm) (Table D8).
- 9. Riviera has darker green leaves than Guymon, Yukon, Arizona Common, and NuMex Sahara (Table D9)

- 10. Mean leaf width of Riviera (2.13 mm) is less than that of Guymon (3.29 mm) and greater than that of Arizona Common (1.87 mm) and NuMex Sahara (1.76 mm) (Table D10).
- 11. Mean leaf width of Riviera (2.5 mm) is greater than that of Princess (2.22 mm) (Table D11).
- 12. Mean leaf length of Riviera (47.9 mm) is less than that of Guymon (76.9 mm), and NuMex Sahara (60.4 mm) (Table D12).
- 13. Mean leaf length of Riviera (37.95 mm) is less than that of Princess (18.79 mm) based on measurements of greenhouse grown plants (Table D13).
- 14. Mean flag leaf width of Riviera (1.25 mm) is less than that of Guymon (1.74 mm) and Yukon (1.33 mm), and greater than that of Arizona Common (1.15 mm) and NuMex Sahara (1.05 mm) (Table D14).
- 15. Mean flag leaf length of Riviera (21.7 mm) is less than that of Guymon (40.5 mm), NuMex Sahara (30.2 mm) and Arizona Common (26.7 mm) (Table D15).
- 16. Mean lateral leaf width of Riviera (2.22 mm) is less than that of NuMex Sahara (2.68 mm), Arizona Common (2.63 mm), and Guymon (2.56 mm), and greater than that of Yukon (2.02 mm) (Table D16).
- 17. Mean lateral leaf width of Riviera (2.45 mm) is less than that of Princess (2.21 mm) based on measurements of greenhouse grown plants (Table D17).
- 18. Mean lateral leaf length of Riviera (21.2 mm) is less than that of Yukon (34 mm), Arizona Common (33.4 mm), NuMex Sahara (33.1 mm), and Guymon (25.6 mm) (Table D18).
- 19. Density of leaf hairs on leaf blades, leaf sheaths, and collars is less on Riviera than on Guymon (Table D19).
- 20. Mean inflorescence length of Riviera (45.4 mm) is shorter than that of Guymon (60.7 mm) and NuMex Sahara (48.6 mm) and longer than that of Arizona Common (43.3 mm) (Table D20).
- 21. Mean number of racemes per inflorescence for Riviera (4.2) is less that that of Guymon (5.7), Yukon (5.4), NuMex Sahara (5.1) and Arizona Common (5) (Table D21).
- 22. Mean number of raceme whorls per inflorescence for Riviera (1) is less than that of Yukon (1.06) and Guymon (1.04) (Table D22).

- 23. Mean number of spikelets per raceme for Riviera (41.1) is less than that of Guymon (51.6) and NuMex Sahara (43.2) and greater than that of Arizona Common (39.0) and Yukon (35.4) (Table D23).
- 24. Rivera has a greater percentage of plants with white stigmas and lower percentages of plants with light purple or purple stigmas than Guymon, NuMex Sahara, Arizona Common, and Yukon. Riviera has a slightly higher percentage of plants bearing yellow anthers and a slightly lower percentage of plants bearing purple anthers than Guymon, NuMex Sahara, and Yukon. Riviera has much higher and lower percentages of plants with yellow and purple anthers, respectively, than Arizona Common (Table D24).
- 25. Mean head exertion length of Riviera (19.1 mm) is less than that of Guymon (39.4 mm) and NuMex Sahara (24.9 mm) and greater than that of Yukon (14.6 mm) (Table D25).
- 26. Mean peduncle length of Riviera (81.3 mm) is less than that of Guymon (133.5 mm) and NuMex Sahara (99.2 mm), and greater than that of Yukon (69.7 mm) (Table D26).
- 27. Mean 1st internode length of seed stalks of Riviera (39.1 mm) is less than that of Guymon (97 mm) and NuMex Sahara (57.4 mm) (Table D27).
- 28. Mean flag leaf sheath length of Riviera (62.2 mm) is less than that of Guymon (97.0 mm) and NuMex Sahara (73.9 mm) and greater than that of Yukon (54.1 mm) (Table D28).
- 29. Mean mature plant height of Riviera (508 mm) is less than that of Guymon (638.8 mm), Arizona Common (604.5 mm), and NuMex Sahara (564.5 mm) and greater than that of Yukon (411.1 mm) (Table D29).
- 30. Mean mature vegetative plant height of Riviera (287 mm) is less than that of Guymon (414 mm), NuMex Sahara (363.2 mm), and Arizona Common 9340.4 mm) (Table D30).
- 31. DNA profiling easily differentiated Riviera from the following seeded varieties: Mirage, Jackpot, Arizona Common, CD90160, Mohawk, Savannah, Southern Star, Sundevil, NuMex Sahara, Sydney, Pyramid, Transcontinental, Majestic, Riviera, Princess, SWI-11, and Yukon (See attached manuscript entitled 'DNA Fingerprinting of Seeded Bermudagrass Cultivars' by Praveen Nagh Yerramsetty, Michael P. Anderson, Charles M. Taliaferro and Dennis L. Martin. The manuscript has been accepted for publication in Crop Science and is in press as of Oct. 2004).

Table D1. Relative freeze tolerance of turf bermudagrass varieties as determined by laboratory analyses. T_{mid} values represent the midpoint of the survival-temperature response curve. Data from: Anderson, J, C. Taliaferro, D. Martin. Longer exposure durations increase freeze damage to turf bermudagrasses. Crop Sci. (In press).

Variety	Type	T _{mid} (°C)
Tifway	Clonal	-7.9 b*
TifSport	Clonal	-7.9 b
Midlawn	Clonal	-10.3 e
U-3	Clonal	-8.9 cd
Patriot	Clonal	-9.7 de
Princess	Seeded	-6.9 a
Riviera	Seeded	-8.3 be

^{*}Means within a column followed by the same letter are not significant at the 0.05 probability level as determined by Duncan's Multiple Range test.

Table D2. Mean ratings for varieties in the National Turfgrass Evaluation Program bermudagrass test-1997 for traits related to winter hardiness.¹

	Spring Greenup ²	% Living Ground	% Winterkill ⁴	Winter Injury
Variety		Cover (Spring) ³		Ratings ⁵
variety	Seede	d Varieties	L	1
Riviera	6.3	90.4	18.1	7.0
Princess	5.0	67.3	62.4	2.3
SWI-11	3.8	63.8	68.8	2.0
Transcontinental	5.2	77.6	41.3	4.3
Savannah	4.6	64.9	64.6	2.3
Southern Star	4.7	67.7	50.3	3.0
Blackjack	4.7	74.0	26.9	4.7
J-540	4.4	59.9	54.4	2.7
Majestic	3.9	58.2	66.7	1.7
Sydney	4.3	63.7	57.9	1.3
Sundevil II	4.4	64.0	57.0	3.0
Shangri La	4.1	57.6	58.2	1.7
Pyramid	4.2	58.6	59.7	2.0
Blue-Muda	4.4	58.4	57.3	1.7
Mirage	4.1	60.7	43.3	1.7
Numex-Sahara	4.1	54.1	51.8	1.7
Jackpot	4.3	58.7	58.7	2.0
Arizona Common	3.7	45.2	50.0	1.7
LSD 0.05	.03	6.9	12.2	1.3
CV (%)	35.8	37.3	32.3	31.5
	Vegetat	ive Varieties		
TifSport	4.8	71.4	35.4	3.7
Tifway	5.0	76.8	41.4	2.7
Tifgreen	5.5	78.7	43.5	4.0
Patriot	5.4	84.0	15.5	6.7
CN 2-9	5.0	70.1	34.7	2.3
Midlawn	6.1	85.9	14.8	5.7
OKC 19-9	5.9	79.9	11.8	6.3
Shanghai	5.3	77.8	29.8	5.3
Mini-Verde	4.3	59.3	62.7	3.0
Cardinal	6.3	87.0	11.5	5.0
LSD 0.05	0.3	5.5	10.4	2.3
CV (%)	32.8	24.8	47.9	32.7

¹Source: Morris, K. N. 2002. National Bermudagrass Test- 1997, Final Report 1997-2001, NTEP No. 02-7. National Turfgrass Evaluation Program, Beltsville Agricultural Research Center-West, Bldg. 003, Rm 218, Beltsville, MD. ²Mean of 14 locations. ³Mean of 14 locations. ⁴Mean of four northern locations, Fayetteville, AR, Wichita, KS, Columbia, MO, and Stillwater, OK. ⁵Winter injury ratings at Wichita, KS. Ratings on a scale of 1 to 9, 9=no injury.

Table D3. Mean (1997-2001) turfgrass quality ratings of bermudagrass varieties in the National Turfgrass Evaluation Program bermudagrass test – 1997. Ratings are on a scale of 1 to 9, with 9 representing ideal turf quality.*

	Management Schedule ²		
Variety	A	В	Avg.
	Seeded Var	ieties	
Riviera	6.4	6.6	6.5
Princess	6.5	6.1	6.3
SWI-11	6.1	5.5	5.8
Transcontinental	6.0	5.6	5.7
Savannah	5.4	5.3	5.3
Southern Star	5.4	5.4	5.3
Blackjack	5.3	5.4	5.2
J-540	5.3	5.2	5.2
Majestic	5.3	5.3	5.2
Sydney	5.2	5.2	5.2
Sundevil II	5.3	5.0	5.1
Shangri La	5.2	5.1	5.1
Pyramid	5.2	5.0	5.0
Blue-Muda	5.1	5.0	5.0
Mirage	5.1	4.9	5.0
Numex-Sahara	5.0	5.0	4.9
Jackpot	5.0	4.9	4.9
Arizona Common	4.7	4.5	4.6
LSD 0.05	0.2	0.2	0.1
CV (%)	12.7	15.4	14.5
	Vegetative Va	rieties	· .
TifSport	6.5	6.1	6.4
Tifway	6.4	6.2	6.3
Tifgreen	6.1	6.3	6.2
Patriot	6.1	6.6	6.3
CN 2-9	6.1	5.8	5.9
Midlawn	5.8	6.5	6.1
OKC 19-9	5.8	6.1	5.9
Shanghai	5.6	6.1	5.9
Mini-Verde	5.6	5.1	5.4
Cardinal	5.4	5.7	5.5
		4	
LSD 0.05	0.2	0.2	0.1
CV (%)	15.8	14.2	14.5

*Source: Morris, K. N. 2002. National Bermudagrass Test- 1997, Final Report 1997-2001, NTEP No. 02-7. National Turfgrass Evaluation Program, Beltsville Agricultural Research Center-West, Bldg. 003, Rm 218, Beltsville, MD.

²Schedule A= ½ to ¾ inch mowing height, ¾ to 1 lb N/1000 ft²/growing month, irrigation to prevent visual drought stress, and mowing frequency 3 to 5 times/week. Data from nine test locations. Schedule B= ¾ to 1 inch mowing height, ½ to ¾ lb N/1000 ft²/growing month, irrigation to prevent dormancy, and mowing frequency 1-2 times/week. Data from 12 test locations.

Table D4. Mean stolon internode diameter for seven field grown seed-propagated turf bermudagrass varieties based on five measurements from each of 60 plants. Measurements taken from center of 3rd fully extended internode from the apical meristem. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

Variety	Stolon Internode Diameter		
	Mean	Range	
	mn	n	
Guymon	2.04 a*	1.02 - 2.61	
Mirage	1.38 b	1.02 - 2.29	
Yukon	1.35 b	0.76 - 2.03	
Arizona Common	1.33 b	0.76 - 2.03	
NuMex Sahara	1.28 bc	0.76 - 1.79	
Jackpot	1.19 bc	0.76 - 1.79	
Riviera	1.11c	0.76 - 1.79	

^{*}Means followed by the same letter are not statistically different based on the 5% LSD.

Table D5. Mean stolon internode length for seven field grown seed-propagated bermudagrass varieties based on five measurements from each of 60 plants. Measured at 3rd fully extended internode from the meristem. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

Variety	Stolon Internode Length		
	Mean	Range	
-	mr	n	
Arizona Common	46.70 a*	12.00 - 159.00	
NuMex Sahara	43.99 ab	13.00 - 89.00	
Riviera	41.98 bc	10.00 - 79.00	
Mirage	39.65 c	10.00 - 114.00	
Guymon	34.95 d	8.00 - 125.00	
Yukon	31.55 d	8.00 - 105.00	
Jackpot	27.86 e	6.00 - 73.00	

Table D6. Mean number of growing points emanating from the 4th node of mature stolons of seven field grown seed-propagated turf bermudagrasses based on five measurements from each of 60 plants. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

Variety	Growing Points/4th Node		
	Mean	Range	
	No		
Mirage	1.37 a*	1.00 - 3.00	
NuMex Sahara	1.32 a	1.00 - 2.00	
Rivera	1.31 a	1.00 - 2.00	
Arizona Common	1.21 b	1.00 - 2.00	
Jackpot	1.18 bc	1.00 - 2.00	
Yukon	1.16 bc	1.00 - 2.00	
Guymon	1.09 c	1.00 - 2.00	

Table D7. Mean number of growing points emanating from the 4th node of mature stolons of 'Princess' and 'Riviera' seed-propagated turf bermudagrasses based on five measurements from each of 60 plants grown in the greenhouse. Okla. St. Univ. Agron. Res. Stn., Stillwater, OK. 2002.

Variety	Growing Points/4 th Node	
•	Mean	Range
	No	
Princess	1.56 a	0.00 - 7.00
Riviera	1.26 b	0.85 - 4.00

Table D8. Mean stolon length for seven field grown seed-propagated bermudagrass varieties based on five measurements from each of 60 plants. Stolon length measured from the apical meristem to the 5th node. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

Variety	Stolon length		
	Mean	Range	
	mı	m	
Arizona Common	215.6 a*	99 – 582	
NuMex Sahara	206.2 ab	108 – 363	
Riviera	194.9 вс	79 - 373	
Mirage	187.9 с	69 – 425	
Guymon	171.1 d	73 – 390	
Yukon	146.3 e	60 - 363	
Jackpot	145.9 e	82 - 270	

Table D9. Mean leaf blade color ratings for seven field grown seed-propagated bermudagrass varieties based on five ratings from each of 60 plants. Rating scale was 1 = light green; 9 = dark green. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

Variety	Rating			
-	Mean	Range		
Riviera	8.4 a	6-9		
Guymon	7.7 b	7 – 9		
Yukon	7.4 b	6 – 9		
Mirage	6.5 c	5 – 9		
Jackpot	6.4 c	6 – 7		
Arizona Common	6.3 с	5 – 7		
NuMex Sahara	5.6 d	3 - 7		

Table D10. Mean leaf width for seven field grown seed-propagated bermudagrass varieties based on five measurements from each of 60 plants. Leaf width was measured on first fully extended leaf of upright growth at widest part. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

Variety	Leaf width			
	Mean	Range		
	mm			
Guymon	3.29 a*	2.03 - 5.33		
Yukon	2.36 b	1.27 - 3.30		
Riviera	2.13 c	1.52 – 3.05		
Mirage	2.07 с	1.27 - 2.94		
Arizona Common	1.87 d	1.27 - 2.54		
NuMex Sahara	1.76 e	1.27 - 2.54		
Jackpot	1.56 f	1.01 - 2.79		

Table D11. Mean leaf width for 'Princess' and 'Riviera' seed-propagated bermudagrass varieties based on five measurements from each of 20 greenhouse grown plants. Leaf width was measured on first fully extended leaf of upright growth at widest part. Okla. St. Univ. Agron. Res. Stn., Stillwater, OK. 2002.

Variety	Leaf width		
	Mean	Range	
	mm		
Princess	2.22 a*	1.00 - 3.00	
Riviera	2.50 b	1.80 = 3.10	

Table D12. Mean leaf length for seven field grown seed-propagated bermudagrass varieties based on five measurements from each of 60 plants. Leaf length was measured on first fully extended leaf of upright growth. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

Variety	Leaf length			
	Mean	Range		
	mm			
Guymon	76.9 a*	26 – 214		
NuMex Sahara	60.4 b	17 - 135		
Arizona Common	52.2 c	17 - 120		
Mirage	49.4 cd	15 - 110		
Riviera	47.9 cde	25 - 93		
Yukon	47.1 de	18 - 75		
Jackpot	43.7 e	12 - 109		

Table D13. Mean leaf length for 'Princess' and 'Riviera' seed-propagated bermudagrass varieties based on five measurements from each of 20 greenhouse grown plants. Leaf length was measured on first fully extended leaf of upright growth. Okla. St. Univ. Agron. Res. Stn., Stillwater, OK. 2002.

Variety	Leaf length			
	Mean Range			
	mm			
Princess	18.79 a*	2.00 - 99.00		
Riviera	37.95 b	9.00 - 115.00		



Table D14. Mean flag leaf width for seven field grown seed-propagated bermudagrass varieties based on five measurements from each of 60 plants. Width measured at widest part of leaf. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

Variety	Flag leaf width			
	Mean	Range		
	mm			
Guymon	1.74 a*	0.76 - 3.81		
Yukon	1.33 b	0.76 - 2.03		
Riviera	1.25 c	0.76 - 2.03		
Arizona Common	1.15 d	0.76 - 2.03		
Mirage	1.14 d	0.51 - 2.29		
NuMex Sahara	1.05 e	0.76 - 1.78		
Jackpot	0.99 e	0.76 - 2.03		

Table D15. Mean flag leaf length for seven field grown seed-propagated bermudagrass varieties based on five measurements from each of 60 plants. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

Variety	Flag leaf length			
	Mean	Range		
	mm			
Guymon	40.5 a*	5 – 144		
NuMex Sahara	30.2 b	5 – 90		
Arizona Common	26.7 c	5 – 65		
Mirage	24.9 cd	5 – 93		
Jackpot	23.1 de	4 - 80		
Riviera	21.7 de	6-50		
Yukon	20.3 e	6 - 61		

Table D16. Mean lateral leaf width for seven field grown seed-propagated bermudagrass varieties based on five measurements from each of 60 plants. Measurements taken at widest part of the first fully extended leaf from tip of stolon. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

Variety	Lateral le	eaf width	
	Mean	Range	
	mm		
NuMex Sahara	2.68 a*	1.52 - 3.81	
Arizona Common	2.63 ab	1.78 - 3.30	
Mirage	2.59 ab	1.52 - 3.56	
Guymon	2.56 ab	0.76 - 5.33	
Jackpot	2.55 b	1.01 - 3.81	
Riviera	2.22 c	1.27 – 3.04	
Yukon	2.02 d	0.76 - 3.81	

Table D17. Mean lateral leaf width for 'Princess' and 'Riviera' seed-propagated bermudagrass varieties based on five measurements from each of 60 greenhouse grown plants. Measurements taken at widest part of the first fully extended leaf from tip of stolon. Okla. St. Univ. Agronomy Res. Stn., 2002.

Variety	Lateral leaf width			
·	Mean	Range		
	mm			
Princess	2.21 a*	1.00 - 3.00		
Riviera	2.45 b	2.00 - 3.10		

Table D18. Mean lateral leaf length for seven field grown seed-propagated bermudagrass varieties based on five measurements from each of 60 plants. Measured the first fully extended leaf from tip of stolon. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

Variety	Lateral leaf length		
	Mean	Range	
	mı	m	
Yukon	34.0 a*	3.0 - 126.2	
Arizona Common	33.4 a	16.5 - 58.9	
NuMex Sahara	33.1 a	9.4 – 91.2	
Mirage	31.6 a	11.9 – 73.7	
Guymon	25.6 b	5.3 – 128.8	
Jackpot	25.3 b	10.2 - 64.0	
Rivera	21.2 c	7.6 – 45.5	

Table D19. Mean ratings of leaf hair density for seven field grown seed-propagated bermudagrass varieties based on five measurements from each of 60 plants. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000. Rating scale: 1= least dense hairs, 9=most dense hairs.

	Leaf	Blade	She	eath	Co	llar
Variety	Mean	Range	Mean	Range	Mean	Range
Guymon	5.9 a*	1 – 9	3.8 a	1 – 6	4.6 a	1 - 7
Riviera	1.5 b	1-6	1.8 b	1-6	2.3 b	1-6
Arizona	1.5 b	1 – 5	1.9 b	1 – 6	2.5 b	1 – 7
Common						
Yukon	1.4 b	1 – 5	1.8 b	1 - 7	2.4 b	, 1 - 6
NuMex	1.4 b	1 - 2	1.7 b	1 - 6	2.1 b	1 - 6
Sahara	,	· ·				
Mirage	1.3 b	1-2	1.6 b	1-6	2.2 b	1-6
Jackpot	1.3 b	1 - 3	1.5 b	1 – 5	2.1 b	1-6

Table D20. Mean inflorescence length for seven seed-propagated turf bermudagrass varieties based on measurements from five inflorescences from each of 60 plants. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

Variety	Inflorescence length		
	Mean	Range	
	mr	n	
Guymon	60.7 a*	29 – 84	
NuMex Sahara	48.6 b	25 - 75	
Riviera	45.4 c	30 - 62	
Mirage	44.8 cd	25 - 70	
Yukon	43.8 cd	15 - 67	
Jackpot	43.6 cd	20 - 65	
Arizona Common	43.3 d	24 - 70	

Table D21. Mean number of racemes per inflorescence for seven field grown seed-propagated bermudagrass varieties based on measurements from five inflorescences from each of 60 plants. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

Variety	Number of racemes/inflorescence		
•	Mean	Range	
	Nu1	Number	
Guymon	5.7 a*	3 – 9	
Yukon	5.4 b	3 – 9	
NuMex Sahara	5.1 c	4 – 6	
Jackpot	5.0 с	3 – 6	
Arizona Common	5.0 с	3 – 8	
Mirage	5.0 c	4-7	
Riviera	4.2 d	3-5	

Table D22. Mean number of raceme whorls per inflorescence for seven field grown seed-propagated bermudagrass varieties based on measurements from five inflorescences from each of 60 plants. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

Variety	Number of raceme w	horls/inflorescence
·	Mean	Range
	Num	nber
Yukon	1.06 a*	1 – 2
Guymon	1.04 ab	1 – 2
Arizona Common	1.03 abc	1 – 2
Mirage	1.02 bc	1 – 2
Jackpot	1.00 c	1 – 1
NuMex Sahara	1.00 с	1 - 1
Riviera	$1.00\mathrm{c}$	

Table D23. Mean number of spikelets per raceme for seven field grown seed-propagated bermudagrass varieties based on measurements from five inflorescences from each of 60 plants. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

Variety	Number of spikelets/ raceme		
	Mean	Range	
-	Number		
Guymon	51.6 a*	24 – 74	
NuMex Sahara	43.2 b	20 – 65	
Jackpot	42.3 bc	17 – 63	
Mirage	41.3 c	16 – 67	
Riviera	41.1 c	24 - 57	
Arizona Common	39.0 d	24 – 63	
Yukon	35.4 e	14 - 53	

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Table D24. Mean percentage plants based on stigma and anther color for seven field grown seed-propagated bermudagrass varieties based on measurements from five inflorescences from each of 60 plants. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

S		Stigma color		Anther color	
Variety		Light			
	White	Purple	Purple	Yellow	Purple
-	% if plants				
Rivera	90	5	5	80	20
Guymon	10	20	70	75	25
NuMex Sahara	40	35	25	65	35
Jackpot	50	30	20	100	0
Mirage	10	20	70	65	35
Arizona Common	15	35	50	15	85
Yukon	75	10	15	90	10

Table D25. Mean head exsertion length for seven field grown seed-propagated bermudagrass varieties based on measurements from five inflorescences from each of 60 plants. Measurements from base of inflorescence to the flag leaf. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

Variety	Head exsertion length		
	Mean	Range	
	mm		
Guymon	39.4 a*	1-107	
Jackpot	33.3 b	0 – 96	
Mirage	26.3 с	0 - 84	
NuMex Sahara	24.9 c	0 – 97	
Arizona Common	22.3 cd	0 - 78	
Riviera	19.1 d	0-79	
Yukon	14.6 e	0 - 63	



Table D26. Mean peduncle length for seven field grown seed-propagated bermudagrass varieties based on measurements from five inflorescences from each of 60 plants. Measurements from base of whorl to first node. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

Variety	Mean peduncle length	
	Mean	Range
	mm	
Guymon	133.5 a*	16 – 231
NuMex Sahara	99.2 b	58 - 182
Jackpot	93.2 с	11 - 165
Mirage	91.4 cd	47 - 162
Arizona Common	86.3 de	47 – 156
Riviera	81.3 e	16 - 86
Yukon	69.7 f	18 - 122

Table D27. Mean first internode length of seed stalks for seven field grown seed-propagated bermudagrass varieties based on measurements from five shoots from each of 60 plants. Measurements made on first internode below inflorescence. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

Variety	First internode length		
	Mean	Range	
_	mr	mm	
Guymon	97.0 a*	20 –232	
NuMex Sahara	57.4 b	27 – 120	
Jackpot	50.8 c	15 - 80	
Mirage	50.7 с	25 – 93	
Arizona Common	42.1 d	19 – 83	
Riviera	39.1 de	16 - 86	
Yukon	37.0 e	13 - 69	

Table D28. Mean flag leaf sheath length for seven field grown seed-propagated bermudagrass varieties based on measurements from five seed stalks from each of 60 plants. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

Variety	Flag leaf sh	eath length
	Mean	Range
-	mm	
Guymon	97.0 a*	51 – 164
NuMex Sahara	73.9 b	46 – 139
Mirage	64.8 c	40 – 93
Arizona Common	63.8 cd	29 – 107
Riviera	62.2 cd	40 = 87
Jackpot	60.8 d	24 – 123
Yukon	54.1 e	15 - 80

*Means followed by the same letter are not statistically different based on the 5% LSD.

Table D29. Mean mature plant height for seven field grown seed-propagated bermudagrass varieties based on five measurements from each of 60 plants. Measurements made by selecting tallest seed stalks and measuring from soil line to tip of inflorescence. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

Variety	Plant	height					
	Mean	Range					
	mm						
Guymon	638.8 a*	406 - 787					
Arizona Common	604.5 ab	432 – 787					
NuMex Sahara	564.5 bc	381 – 711					
Mirage	551.2 cd	356 – 762					
Riviera	508.0 d	356 - 737					
Jackpot	422.3 e	229 – 572					
Yukon	411.1 e	127 - 533					

Means followed by the same letter are not statistically different based on the 5% LSD.

Table D30. Mean mature vegetative plant height for seven field grown seed-propagated bermudagrass varieties based on five measurements from each of 60 plants. Measurements made from soil line to top of vegetative canopy. Okla. St. Univ. Agronomy Res. Stn., Stillwater, OK. 2000.

Variety	Vegetative plant height						
-	Mean	Range					
	mn	1					
Guymon	414.0 a*	279 – 533					
NuMex Sahara	363.2 b	304 – 432					
Mirage	348.0 b	178 - 457					
Arizona Common	340.4 b	203 - 559					
Jackpot	297.2 с	152 - 381					
Yukon	292.1 с	51 – 483					
Riviera	287.0 c	229 - 432					

*Means followed by the same letter are not statistically different based on the 5% LSD.

Table B1. Mean ratings for varieties in the National Turfgrass Evaluation Program bermudagrass test-1997 for traits related to winter hardiness.¹

	Spring Greenup ²	% Living Ground Cover	% Winterkill ⁴	Winter Injury Ratings ⁵				
Variety	Seede	(Spring) ³ 1 Varieties						
Riviera	6.3		18.1	7.0				
Princess	5.0	67.3	62.4	2.3				
SWI-11	3.8	63.8	68.8	2.0				
Transcontinental	5.2	77.6	41.3	4.3				
Savannah	4.6	64.9	64.6	2.3				
Southern Star	4.7	67.7	50.3	3.0				
Blackjack	4.7	74.0	26.9	4.7				
J-540	4.4	59.9	54.4	2.7				
Majestic	3.9	58.2	66.7	1.7				
Sydney	4.3	63.7	57.9	1.3				
Sundevil II	4.4	64.0	57.0	3.0				
Shangri La	4.1	57.6	58.2	1.7				
Pyramid	4.2	58.6	59.7	2.0				
Blue-Muda	4.4	58.4	57.3	1.7				
Mirage	4.1	60.7	43.3	1.7				
Numex-Sahara	4.1	54.1	51.8	1.7				
Jackpot	4.3	58.7	58.7	2.0				
Arizona Common	3.7	45.2	50.0	1.7				
LSD 0.05	.03	6.9	12.2	1.3				
CV (%)	35.8	37.3	32.3	31.5				
		ve Varieties		1				
TifSport	4.8	71.4	35.4	3.7				
Tifway	5.0	76.8	41.4	2.7				
Tifgreen	5.5	78.7	43.5	4.0				
Patriot	5.4	84.0	15.5	6.7				
CN 2-9	5.0	70.1	34.7	2.3				
Midlawn	6.1	85.9	14.8	5.7				
OKC 19-9	5.9	79.9	11.8	6.3				
Shanghai	5.3	77.8	29.8	5.3				
Mini-Verde	4.3	59.3	62.7	3.0				
Cardinal	6.3	87.0	11.5	5.0				
LSD 0.05	0.3	5.5	10.4	2.3				
CV (%)	32.8	24.8	47.9	32.7				

¹Source: Morris, K. N. 2002. National Bermudagrass Test-1997, Final Report 1997-2001, NTEP No. 02-7. National Turfgrass Evaluation Program, Beltsville Agricultural Research Center-West, Bldg. 003, Rm 218, Beltsville, MD. ²Mean of 14 locations. ³Mean of 14 locations. ⁴Mean of four northern locations, Fayetteville, AR, Wichita, KS, Columbia, MO, and Stillwater, OK. ⁵Winter injury ratings at Wichita, KS. Ratings on a scale of 1 to 9, 9=no injury.

Table B2. Mean (1997-2001) turfgrass quality ratings of bermudagrass varieties in the National Turfgrass Evaluation Program bermudagrass test – 1997. Ratings are on a scale of 1 to 9, with 9 representing ideal turf quality.*

	Manageme		
Variety	A	В	Avg.
	Seeded Var	ieties	
Riviera	6.4	6.6	6.5
Princess	6.5	6.1	6.3
SWI-11	6.1	5.5	5.8
Transcontinental	6.0	5.6	5.7
Savannah	5.4	5.3	5.3
Southern Star	5.4	5.4	5.3
Blackjack	5.3	5.4	5.2
J-540	5.3	5.2	5.2
Majestic	5.3	5.3	5.2
Sydney	5.2	5.2	5.2
Sundevil II	5.3	5.0	5.1
Shangri La	5.2	5.1	5.1
Pyramid	5.2	5.0	5.0
Blue-Muda	5.1	5.0	5.0
Mirage	5.1	4.9	5.0
Numex-Sahara	5.0	5.0	4.9
Jackpot	5.0	4.9	4.9
Arizona Common	4.7	4.5	4.6
LSD 0.05	0.2	0.2	0.1
CV (%)	12.7	15.4	14.5
	Vegetative Va	rieties	
TifSport	6.5	6.1	6.4
Tifway	6.4	6.2	6.3
Tifgreen	6.1	6.3	6.2
Patriot	6.1	6.6	6.3
CN 2-9	6.1	5.8	5.9
Midlawn	5.8	6.5	6.1
OKC 19-9	5.8	6.1	5.9
Shanghai	5.6	6.1	5.9
Mini-Verde	5.6	5.1	5.4
Cardinal	5.4	5.7	5.5
	ν		
LSD 0.05	0.2	0.2	0.1
CV (%)	15.8	14.2	14.5

*Source: Morris, K. N. 2002. National Bermudagrass Test- 1997, Final Report 1997-2001, NTEP No. 02-7. National Turfgrass Evaluation Program, Beltsville Agricultural Research Center-West, Bldg. 003, Rm 218, Beltsville, MD.

²Schedule  $A = \frac{1}{2}$  to  $\frac{3}{4}$  inch mowing height,  $\frac{3}{4}$  to 1 lb N/1000 ft²/growing month, irrigation to prevent visual drought stress, and mowing frequency 3 to 5 times/week. Data from nine test locations. Schedule  $B = \frac{3}{4}$  to 1 inch mowing height,  $\frac{1}{2}$  to  $\frac{3}{4}$  lb N/1000 ft²/growing month, irrigation to prevent dormancy, and mowing frequency 1-2 times/week. Data from 12 test locations.

1	DNA Fingerprinting of Seeded Bermudagrass Cultivars
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#### **ABSTRACT**

2 3

Bermudagrasses (Cynodon spp.) are important for turf and forage in temperate and tropical climates, 4 with cultivars historically propagated clonally. Over the past two decades the number of seed-5 propagated commercial cultivars has dramatically increased, but information is lacking on the extent of the genetic diversity among these new cultivars. Accordingly, this research was undertaken to assess the 6 7 genetic relatedness of 17 seed-propagated turf-bermudagrass cultivars using DNA amplification fingerprinting (DAF). Four DAF and four Minihairpin-DAF (MHP-DAF) primers were used in this 8 9 study. The DAF and MHP-DAF primers amplified 90 and 131 amplicons, respectively. A total of 13 out of the 17 cultivars were practically indistinguishable using the DAF primers with an average similarity 10 11 (SC) of 0.982, while the MHP-DAF primers distinguished all cultivars readily. Results from the DAF and MHP-DAF analysis indicated that 14 out of the 17 cultivars were related to Arizona common 12 germplasm with average SC of 0.833 in the MHP-DAF analysis. Arizona common germplasm is 13 naturalized to the Colorado River Valley production areas of Arizona and California. The three most 14 distinct cultivars: 'Princess 77', 'Yukon' and 'SWI-11' had an average SC of 0.668. The most distinct 15 cultivar was 'Yukon' with an average SC of 0.604. Yukon showed 59 DNA signatures not observed in 16 17 the other varieties studied with DAF and MHP-DAF. These results indicated that a majority of seededtype bermudagrasses developed over the past two decades depend upon a narrow genetic base, and that 18 19 several recent cultivars are markedly genetically distinct indicating a recent and significant broadening 20 of the germplasm.

Bermudagrass (Cynodon dactylon L. Pers) is a perennial sod-forming turf and forage grass, native to India and eastern Africa (Beard 1973; Braun 1967; Correl and Johnson 1970; Duble 1996). 2 This grass is extensively used in temperate and subtropical regions of the world for agricultural, recreational and residential use (Duble, 1996). Historically, the highest quality turf bermudagrass 4 cultivars have been sterile F₁ hybrid plants from crosses between plants of tetraploid (2n=4x=36) C. dactylon and diploid (2n=2x=18) C. transvalensis Burtt-Davy. These cultivars are commercially propagated by planting either sprigs or sod. Over the past two decades there has been a dramatic increase in the number of seed-propagated cultivars. National Turf Evaluation Program (NTEP) data (NTEP, 2002) indicate some of the recently developed seeded-type bermudagrasses rival the clonalstandard bermudagrass cultivars in turfgrass quality and other performance characteristics.

Several studies have been conducted to examine the genetic relatedness among vegetative propagated bermudagrass cultivars (Caetano-Anolles, 1995 and 1998a; Zhang, 1999), but no information has been published concerning diversity among seeded-type bermudagrasses. Several seeded-type bermudagrass cultivars appear to have originated from the naturalized common form of bermudagrass grown in Yuma County Arizona and the California Imperial Valley and are generally referred to as "Arizona Common". This bermudagrass is thought to have been introduced to the US southwest desert region at least by the middle of the 19th century (Kneebone, 1966). Baltensperger et al. (1993) indicated that a bermudagrass seed industry started soon after 1900 from bermudagrass naturalized to a region along the Colorado river in Arizona and California. The degree to which current commercial seeded-type bermudagrass cultivars are genetically interrelated is unknown. Accordingly, an estimation of genetic diversity of the seeded-type bermudagrass cultivars would provide important information relative to the need for genetic diversification in breeding programs.

Many techniques have been used to determine genetic relationships, including DNA amplification and fingerprinting (DAF) (Caetano- Anolle's et al., 1997), amplified fragment length polymorphism (AFLP)(Zhang et al., 1999), and randomly amplified polymorphic DNA (RAPD) (Huff, 1997). All these take advantage of the natural variations inherent in plant DNA. While all are capable, there are some advantages to each. AFLP is a very powerful and reproducible technique, and is readily adaptable to automation. However the technique is fairly expensive in terms of reagent cost and equipment, and requires additional steps to perform when compared to DAF. The DAF technique is a reliable, low cost, high-resolution method that is capable of revealing many DNA polymorphisms. The DAF method when compared to the similar technique known as RAPD produces a many-fold increase in polymorphism per primer (de Vienne et al., 2003).

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A variant of DAF that utilizes short minihairpin primers further increases the resolving power of the DAF technique. In one study, the MHP primers detected 5 times as many bermudagrass polymorphisms as conventional DAF primers (Caetano-Anolle's et al., 1995). MHP-DAF primers contain palindromic sequences which hybridizes through intra-primer interactions creating a hairpin and a small looped priming structure (Caetano-Anolle's and Gresshoff, 1994). The MHP-DAF technique uses previously amplified DAF amplicons as template to generate further banding pattern diversity.

DAF has been used successfully to determine the phylogenetic relationships among bermudagrass species (Assefa et al., 1999), provide information on the origin of off-type bermudagrass cultivars (Caetano-Anolles, 1998b), and determine the fidelity of bermudagrass commercially sold as 'U-3'(Anderson et al., 2001), a cultivar originally developed in the early 1930's. Accordingly, this project was undertaken with the objective of determining the genetic relatedness of selected seeded-type bermudagrass cultivars. In this study we analyzed 17 seeded cultivars from different backgrounds using DNA amplification fingerprinting.

I

#### MATERIAL AND METHODS

#### **Plant Materials**

The seeds of bermudagrass cultivars were obtained from the suppliers listed in Table 1.

Approximately 4500 seeds of each cultivar were planted in a 15 cm diameter pot containing Metro mix 250 (Scotts-Sierra, Marysville, OH). The high seeding rate was used to insure that the resulting plant populations would be representative of the cultivars. Plants were fertilized with Peters Professional Peat-Lite (Scotts-Sierra, Marysville, OH) and Iron Chelate (Miller Chemical and Fertilizer Corp., Hanover, PA). The plants were fungicide treated with Chlorothalonil: [2,4,5,6-tetrachloroisophthalonitrile] (trade name: Daconil, Ortho group, Columbus, OH) at a rate of 4.2 ml/L and with Aldecarb: [2-Methyl-2-(methylthio)propionaldehydeO-(methylcarbamoyl oxime] (trade name Temik, Union Carbide Inc., NC).

DNA Isolation

A total of two g of leaf tissue was harvested from a single pot containing each cultivar. The leaf tissue was frozen in liquid nitrogen and ground in a mortar and pestle to a fine powder. Genomic DNA was isolated from 100 mg of powdered leaf tissue using the DNeasy plant mini-extraction kit (Qiagen Inc., Valencia CA) according to directions provided by the supplier. The DNA concentration was assessed spectrophotometrically at 260 nm and quality was assessed by the 260/280 ratio (Sambrook et al. 1989). If one or more DNA extracts of the batch of 17 cultivars showed a 260/280 ratio less than 1.8 the entire batch was extracted again. The DNA was suspended to a final concentration of 5 ng/L in 0.5X TE and stored at 4° C. DNA quality was further assessed by TBE agarose gel electrophoresis. All samples showed no sign of DNA degradation.

#### PCR Amplification

Four DAF and four MHP-DAF primers (Table 2) were used to fingerprint the 17 bermudagrass cultivars used in this study. The PCR amplification mixture consisted of 2.5 U of Qiagen *Taq* polymerase (Qiagen Inc., Valencia, CA) 10X PCR buffer which included MgCl₂ for a final concentration of 1.5 mM, 250 μM dNTP, 1.5 μM DAF primers (Integrated DNA Technologies Inc, Corelville, IA), and 0.5 ng of template DNA, with the final volume made to 20 μl with sterile distilled water. The DNA template was initially denatured at 94 °C for 60 seconds. Following denaturation, PCR proceeded at 94° C for 30 seconds, then 30 °C for 30 seconds and 72° C for 30 seconds, cycling back 39 times. A final extension at 72°C for 60 seconds at the end of the 39 cycles was performed. The PCR products were

visualized on a 1% TBE agarose gel impregnated with ethidium bromide at a final concentration of  $0.5 \, \mu \text{g/ml}$ .

The gel was examined to assure that the overall fingerprint intensity was nearly equal among all lanes. If PCR failed to amplify a fingerprint in any one of the 17 reactions then the entire set was re-run until the fingerprints were near equally amplified. Conditions for MHP-DAF were the same as for DAF except that one  $\mu L$  of DAF PCR product was used instead of the genomic DNA template. We also found that adding 6 mM MgCl₂ improved performance of the MHP-DAF.

### **Denaturing Polyacrylamide Electrophoresis**

PCR products were separated on a 20 cm long 6% acrylamide denaturing PAGE gel using a Bio Rad Protean II apparatus (Bio Rad, Richmond CA). The gel was made with Long Ranger Acrylamide (Cambrex Bio Science Inc., Rockland, ME) 1 X TBE and 7.1 M urea. A total of seven µL of PCR products with three µl of loading buffer containing the tracking dye bromphenol blue were mixed and loaded onto the gel. Molecular markers were loaded on either side of the lanes containing the PCR amplicons. Electrophoresis continued at 80 volts until the bromophenol blue strain reached three-quarters of the length of the gel. The gel was removed and stained with silver using a Bioneer silver staining kit (BioNexus, Oakland, CA) according to manufacturer directions. After staining, the gel was equilibrated in 10% (v/v) glycerol and 20 % (v/v) ethanol, covered with cellophane and air dried at room temperature for a week prior to analysis. All 17 PCR products were run on the same gel to facilitate accurate band-to-band comparisions.

#### Data Profiling and Analysis

After silver staining, electrophorectic bands of less than 1.5 kD were scored for their presence (1) or absence (0) for each cultivar. The data were compiled in a Excel spreadsheet and imported into the NTSYS software version 2.0 (Exeter Software, New York, NY) for cluster analysis. Similarity coefficients (SC)(Table 3) were computed by the SIMQUAL module. Cluster analysis was performed according to the unweighted pair group mean algorithm (UPGMA) within the SAHN module of the NTSYS program. The PCR reaction, electrophoresis separation, staining of gels, data profiling and analysis was replicated two to three times. Comparisons showed that there were either no differences, or only very minor differences, between replicate experiments.

#### RESULTS AND DISCUSSION

A total of 90 and 131 bands were scored for DAF and MHP-DAF, respectively (Fig 1). Over 87% (78 bands) and 79% (103 bands) were found to be polymorphic in the bulked samples using DAF and MHP-DAF, respectively, meaning that the band was present in at least one cultivar but was not observed in others.

The DAF results indicated that 13 out of the 17 bermudagrass cultivars were very closely related to each other (Fig. 2a) with an average SC of 0.982 (data not shown). The other four cultivars, Riveria, Princess, SWI1-1 and Yukon were easily distinguishable using DAF. The technique of DAF alone could not resolve differences between Arizona Common and CD 90160 or differences among 'Mohawk', Savannah, Southern Star, 'Sundevil' and 'Numex Sahara' (Fig. 2a, SC = 1.000). In contrast, the MHP-DAF analysis clearly differentiated among all 17 cultivars (Fig. 2b). The differences between DAF and MHP-DAF were even more dramatic with 14 of the most closely related cultivars in the MHP-DAF analysis showing an average SC of 0.833, while in the DAF analysis these same cultivars showed an average SC of 0.975 (data not shown). The results from the MHP-DAF and DAF analysis indicated that 14 of the cultivars in this study were closely related to Arizona Common. This group included Arizona Common, 'CD90160', 'Jackpot', 'Majestic', Savannah, Southern Star, Sundevil, Mohawk, Riviera, 'Mirage', 'Sydney', 'Pyramid', Numex Sahara, and 'Transcontinental'.

According to MHP-DAF analysis, the most closely related cultivars grouped into three clusters, including: Arizona Common and CD90160 (group 1, SC 0.901), Savannah, Southern Star, and Sundevil (group 2, average SC 0.913), and Numex Sahara and Transcontinental (group 3, SC 0.901). The two most similar cultivars were Savannah and Southern Star with a SC of 0.924. The pedigree information available for Savannah (Fraser and Rose-Fricker, 1998) and Southern Star (Samudio and Brede, 2002) indicate that bermudagrass germplasm from Walla Walla, Washington, collected by the respective developers, contributed to the parentage of both cultivars. The use of additional markers may even better differentiate the closely related Arizona Common-type bermudagrasses.

Yukon, Princess 77 and 'SWI-11' were least genetically related to Arizona Common of all the cultivars studied. Futhermore, all three cultivars showed little relationship to each other. Yukon was the most distinct cultivar in this study with an average SC of 0.604 across all cultivars. The least similar cultivar to Yukon was SWI-11 and the most similar was Transcontinental, with SCs of 0.511 and 0.649, respectively. These low SCs indicate that Yukon was the most divergent seeded-type bermudagrass cultivars of those studied. Furthermore, 36 bands from Yukon were not observed in other cultivars tested, and 23 bands were found in all other bermudagrasses studied except Yukon. Combining those

- 1 bands not observed with those uniquely observed in Yukon totalled 59 potential DNA signatures
- 2 representing over 27 % of the bands scored. Yukon is a new cultivar recently released by Oklahoma
- 3 State University. Two other distinct cultivars Princess 77 and SWI-11 had average SCs of 0.689, and
- 4 0.712, respectively. Both Princess 77 and SWI-11 showed 7 signatures not observed in other cultivars in
- 5 the combinded DAF and MHP-DAF studies, or 3% of all bands scored. These DNA signatures may be
- 6 useful for cultivar maintenance and identification purposes.

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The close clustering of the 14 out of 17 cultivars with DAF indicated that most seeded-type bermudagrass cultivars are very closely related. Included in this group is Arizona Common, indicating that many of the cultivars likely originated from breeding populations originally constituted solely, or substantially, from Arizona Common. A second potential reason for some cultivars showing close similarity to Arizona Common relates to mechanical contamination of seed production fields leading to genetic contamination. Seed of many of the cultivars in the study were produced in Yuma Co., Arizona or the Imperial Valley, California where bermudagrass seed production has been concentrated for nearly a century. Preventing the Arizona Common bermudagrass ubiquitous to this region from mechanically contaminating unique cultivar seed production fields and hybridizing with plants of the unique cultivars is difficult. Seed production fields of cultivars that are less well adapted to the region than Arizona Common can quickly be dominated by the latter. Arizona Common growing as an impurity in seed production fields, or growing in adjacent areas, may hybridize with the cultivars resulting in genetic contamination of the desired cultivar. One of the authors (C. M. Taliaferro) has observed seed production fields of cultivars that were less well adapted to the region than Arizona Common become dominated by the latter within 1 to 3 years contingent on the amount of initial contaminent Arizona Common in the stand. Arizona Common growing as contaminant in cultivar seed-production fields, or growing in adjacent areas, has the potential of hybridizing with the cultivars. Hoff (1967) demonstrated natural crossing between Arizona Common and giant bermudagrass (C. dactylon var. aridus), the two major forms of bermudagrass traditionally grown in the region. However, the progeny resulting from the hybridization of tetraploid Arizona Common and diploid giant bermudagrass plants were sterile triploids. Such hybridization between tetraploid cultivars could produce fertile progeny leading to genetic contamination. Relative to the usually sterile vegetatively-propagated bermudagrass cultivars the potential for genetic changes in seeded-type bermudagrass cultivars is greater and warrants additional actions to maintain their genetic fidelity.

It should be noted that significant differences exist among the cultivars grouped with Arizona Common for turf quality, cold tolerance, and other performance traits (National Turfgrass Evaluation

Program, 1997, 2002). Notably, Riviera, though loosely grouped with Arizona Common on the basis of SC values, has much higher turf quality and broader adaptation due to greater cold tolerance. None of the seed-propagated cultivars in the 1992 NTEP trial had turfgrass quality ratings as high as the vegetatively-propagated standard cultivars in the test. Results from the 1997 NTEP bermudagrass test indicated that the development of Princess and Riviera represented a major gain in turfgrass quality for seeded-type bermudagrasses relative to industry-standard clonal cultivars. The development of these two cultivars suggests that major gains in performance can be achieved by breeding in relatively diverse germplams pools with the desired result of maintenance of genetic diversity among cultivars. **ACKNOWLEDGEMENTS** We appreciate the financial support provided by the United States Golf Association. (USGA). We appreciate the technical support of Carole Anderson. Special thanks to Gary Williams, Sharon Williams and Rose Edwards for maintenance of the bermudagrass cultivars at the Oklahoma State University greenhouse. We gratefully acknowledge the support of colleagues Janice Hironaka, James Enis and Madhavi Dhulipala. 

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### **FIGURES**

Figure 1. MHP-DAF electrophoresis gel stained with silver containing PCR amplicons from 17 cultivars of bermudagrass.

Figure 2. DENDROGRAMs from DAF (a) and MHP-DAF (b) analysis of 17 seeded-type bermudagrass cultivars.

## Table 1. Seeded-type bermudagrass cultivars used in this study and their source

Cultivar	Source
Arizona Common	Seeds West, Inc., Roll, AZ
CD 90160	Cebeco International Seeds, Inc., Halsey, OR
Jackpot	Simplot Turf and Horticulture, Boise, ID
Majestic	H &H Seed company Inc., Yuma, AZ
Mirage	Cebeco International Seeds, Inc., Halsey, OR
Mohawk	Seeds West, Inc., Roll, AZ
Pyramid	Cebeco International Seeds Inc., Halsey, OR
Princess 77	Seeds West Inc., Roll, AZ
Riviera	Oklahoma State University, Stillwater, OK
Savannah	Turf Seed Inc, Hubbard, OR
Southern Star	Simplot Turf and Horticulture, Boise, ID
Sundevil	Simplot Turf and Horticulture, Boise, ID
SWI-11	Seeds West Inc., Roll, AZ
Sydney	Seeds West Inc., Roll, AZ
Numex Sahara	Seeds West Inc., Roll, AZ
Transcontinental	Pure Seed Testing, Inc., Hubbard, Or
Yukon	Oklahoma State University, Stillwater, OK

Table 2. Sequence of the DAF and MHP-DAF primers used in this study.

Primer Label	Primer Sequence
DAF 9110	CAGAAACGCC
DAF 9111	GAAACGCC
DAF 9112	GTAACGCC
DAF 9113	GTAACCCC
MHP-DAF 1	GCGAAGCGGA
MHP-DAF 2	GCGAAGCTACG
MHP-DAF 3	GCGAAGCCTA
MHP-DAF 4	GCGACAGCAGA

Дп <mark>к</mark> ои																	1.000
Transcontinental	•															1.000	
Frenk Samun															1.000	0.901	0.611
Sydney														1.000	0.847	0.794	0.626
ll-IMS													1.000	0.718	0.702	0.710	0.511
Sundevil												1.000	0.740	0.809	0.794	0.756	0.603
Southern Star											1.000	906.0	0.786	0.870	0.840	0.832	0.603
Asnnsvs										1.000	0.924	0.908	0.725	0.824	0.794	0.771	0.618
Riviera									1.000	0.824	0.855	0.840	0.733	0.802	0.771	0.779	0.611
TT seeonin9								1.000	0.718	0.695	0.710	0.725	0.756	0.687	0.687	0.649	0.542
Pyramid							1.000	0.718	0.786	0.794	0.840	0.809	0.672	0.802	0.832	0.824	0.595
Mohawk						1.000	0.847	0.733	0.863	0.870	0.885	0.855	0.733	0.817	0.832	0.824	0.626
Mirage					1.000	0.855	0.824	0.710	0.824	0.832	0.878	0.817	0.725	0,855	0.840	0.832	0.603
Majestic				1.000	0.832	0.855	0.824	0.710	0.794	0.863	0.893	0.863	0.725	0.855	0.840	0.786	0.618
дзскрог			1,000	0.870	0.840	0.847	0.786	0.672	0.878	0.855	0.885	0.870	0.733	0.786	0.832	0.855	0.626
CD9010		1.000	0.885	0.878	0.817	0.855	0.809	0.664	0.794	0.847	0.847	0.847	0.695	0.809	_	_	0.603
Arizona Common	ν-	0.901	0.878	0.855	0.809	0.878	0.786	0.656	0.817	0.855	0.870	0.855	0.733	0.786	0.802	0.794	0.611
Varieties	Arizona Common	CD9010	Jackpot	Majestic	Mirage	Mohawk	Pyramid	Princess 77	Riviera	Savannah	Southern Star	Sundevil	SWI-11	Sydney	Numex Sahara	Transcontinental	Yukon

Table 3. Similarity coefficient table (SC) using MHP-DAF analysis

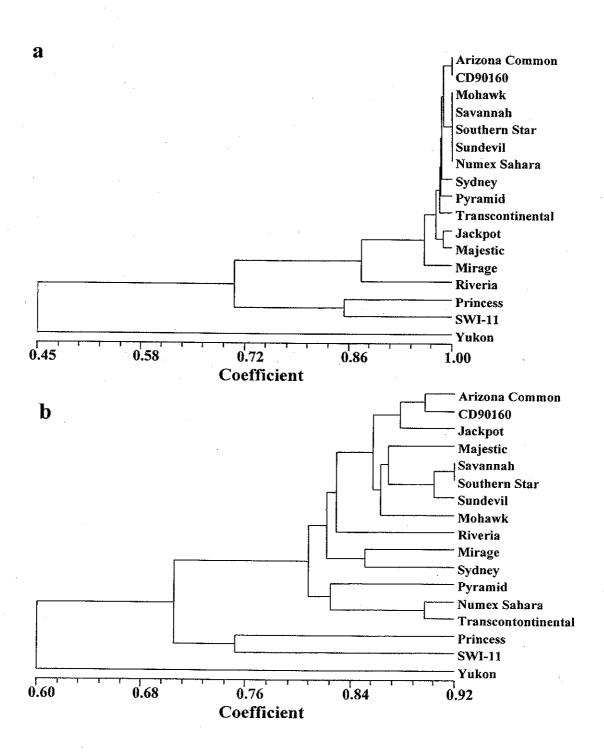


Figure 2. DENDROGRAMs from DAF (a) and MHP-DAF (b) analysis of 17 seeded-type bermudagrass cultivars.

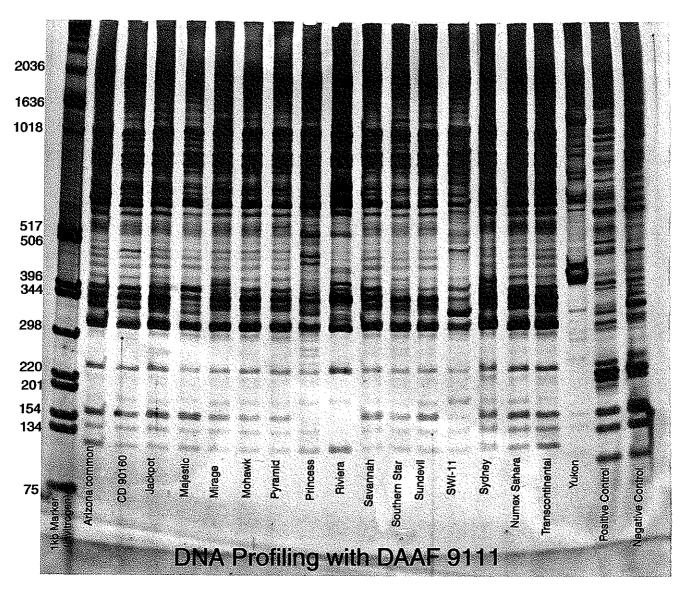


FIG. 1. DAF profiles of 17 seeded turf bermudagrass varieties on a denaturing polyacrylamide gel stained with silver. Polymerase chain reaction (PCR) products were generated from the 9111 primer (GAAACGCC). Molecular marker lane references the nucleic acid fragment size in number of base pairs.

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STATEMENT OF THE BASIS OF OWNERSHIP	confidential until the certificate is issu	ed (7 U.S.C. 2426).					
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4. ADDRESS (Street and No., or R.F.D. No., City, State, and ZIP, and Country)	5. TELEPHONE (Include area code)	6. FAX (include area code)					
Oklahoma State University 139 Agricultural Hall	405-744-5398	405-744-5339					
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8. Does the applicant own all rights to the variety? Mark an "X" in th	l e appropriate block. If no, please expla	in. X YES NO					
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11. Additional explanation on ownership (Trace ownership from original contents of the content	nal breeder to current owner. Use the re	everse for extra space if needed):					
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